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Functional MRI in prenatally opioid-exposed children during a working memory-selective attention task

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ABSTRACT

Background: Opioid induced cerebral changes may contribute to neuropsychological difficulties, like attention problems, frequently reported in prenatally opioid-exposed children. Reduced regional brain volumes have been shown after prenatal opioid exposure, but no study to date has explored the possible impact of prenatal opioids on brain activation patterns.

Materials and methods: A hospital-based sample of prenatally opioid-exposed school-aged children ($n = 11$) and unexposed controls ($n = 12$) underwent functional magnetic resonance imaging (fMRI) during a combined working memory-selective attention task. Within-group- and between-group analyses of blood-oxygen-level-dependent (BOLD) activation were performed using the SPM12 software package and group differences in task performance were analyzed using Cox proportional hazards modeling.

Results: Overall, similar patterns of task related parietal and prefrontal BOLD activations were found in both groups. The opioid-exposed group showed impaired task performance, and during the most cognitive demanding versions of the working memory-selective attention task, increased activation in prefrontal cortical areas was found in the opioid-exposed group compared to controls.

Conclusion: Our findings suggest that prenatal opioids affect later brain function, visible through changes in BOLD activation patterns. However, results should be considered preliminary until replicated in larger samples better suited to control for potential confounding factors.

1. Introduction

Recent studies on the prevalence of neonatal abstinence syndrome (NAS), a common consequence of prenatal opioid exposure, indicate a worldwide increase in the number of children exposed to opioids in utero (Davies et al., 2016; Patrick et al., 2012). Consequences of pre natal opioid exposure beyond NAS are still debated (Mactier et al., 2014; Jones et al., 2014) and the research base is scarce, especially when it comes to possible long term effects (Behnke and Smith, 2013). A web of interconnected risk factors complicates the interpretation of repeatedly reported suboptimal neurocognitive outcomes in prenatally opioid exposed children (Hunt et al., 2008; Nygaard et al., 2015; Ornoy et al., 2001).

Both cognitive and behavioral effects of prenatal opioid exposure have been demonstrated in animal models (Chen et al., 2015; Wang and Han, 2009). Possible mechanisms underlying altered brain function, as suggested by cell culture and animal studies, include increased apop tosis of neurons and glia cells (Hu et al., 2002), altered neuronal dif ferentiation (Dholakiya et al., 2016), decreased neurogenesis (Wu et al., 2014), and altered myelination (Vestal Laborde et al., 2014). Both opioid receptors and opioid ligands are expressed in the fetal brain, and there is growing evidence of the endogenous opioid system as a reg ulator of neurogenesis, with inhibitory effects of opioids (Sargeant et al., 2008). Opioids can affect several neurotransmitters in the

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; BOLD, blood-oxygen-level-dependent; EPI, echo planar imaging; fMRI, functional magnetic resonance imaging; FASD, fetal alcohol spectrum disorder; FWE, family-wise error; HR, hazard ratio; MNI, Montreal Neurological Institute; NAS, neonatal abstinence syndrome; OMT, opioid maintenance treatment

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developing brain, and alterations in neurotransmission could possibly interfere with cognitive development in areas like memory, executive function, and attention (Konijnenberg and Melinder, 2011). However, it is still unclear whether negative effects of opioids on the developing fetal brain contribute to the neuropsychological impairments observed in prenatally exposed children.

Results from a recent longitudinal brain imaging study suggested that several early life factors have an impact on the brain and cognition for the entire life course (Walhovd et al., 2016). Neuroimaging studies have made important contributions to our understanding of how pre natal drug exposures can affect normal brain development, and evi dence of brain structures and patterns of functional activation being altered in exposed children is accumulating (Roussotte et al., 2010; Derauf et al., 2009). However, very few studies have investigated possible brain alterations after prenatal opioid exposure. Structural brain changes in opioid exposed children have been reported in a few small scale samples, including one report on ten children born to mo thers with histories of heroin abuse during pregnancy that showed subtle alterations in structures involved in frontal striatal circuitry (Walhovd et al., 2007). Confounding factors difficult to account for in such small samples preclude firm conclusions, but some of the changes could still be linked to attentional difficulties in the exposed group (Walhovd et al., 2007). To date, no studies have examined possible effects of prenatal opioid exposure on brain activation patterns.

The aim of the present study was to investigate brain activation patterns in school aged children with prenatal opioid exposure using functional magnetic resonance imaging (fMRI). In children with pre natal drug exposure, very high rates of attention deficit/hyperactivity disorder (ADHD) have been reported, regardless of the type of drug exposure (Elgen et al., 2007), and increased risk of attention problems and ADHD has been widely reported in prenatally opioid exposed groups (Ornoy et al., 2001; Ornoy et al., 2016; Sundelin Wahlsten and Sarman, 2013; Nygaard et al., 2016). Associations between attention problems and prenatal opioid exposure have also been found in studies trying to account for the impact of genetic vulnerabilities and postnatal environmental influences (Ornoy et al., 2001; Ornoy et al., 2016). Ex ecutive dysfunction is regarded as a key factor in the complex neu ropsychology of ADHD (Willcutt et al., 2005), and impaired executive functions have been demonstrated in children with prenatal opioid exposure (Konijnenberg and Melinder, 2015). In the present study, a task combining working memory and selective attention was chosen. These are executive functions crucial for normal cognitive function, and most likely implicated in the neurodevelopmental impairments re ported in prenatally opioid exposed children. We hypothesized that prenatally opioid exposed children would show impaired task perfor mance with corresponding differences in blood oxygen level dependent (BOLD) activation as compared with unexposed controls.

2. Materials and methods

2.1. Participants

The opioid exposed group was derived from a larger group of children with prenatal drug exposure referred to the pediatric depart ment at Haukeland University Hospital in Bergen, Norway, between 1997 and 2012. A total of 70 children, aged 10 14 years, identified as prenatally drug exposed, were invited to undergo an MRI examination, as previously described (Sirnes et al., 2017). Children were identified as prenatally drug exposed if they had been admitted to the neonatal department due to maternal drug use, in most cases treated for with drawal symptoms, or if they were referred to a pediatric neurologist at a later age with a medical history of prenatal drug exposure and symp toms of attention and/or behavioral problems. Among the initial 43 children who agreed to participate, 20 children were exposed to opioids, either from heroin abuse or from opioids given as part of opioid maintenance treatment (OMT), and were therefore included in the present study. Information regarding exposure was based on history, but given the presence of heavy substance abuse, detailed information about the frequency or amounts of opioids and the type of other drugs used during pregnancy was not readily available. However, children were only included in the study if prenatal opioid exposure could be confirmed, either in medical records (obstetric or pediatric) or by in formation from their mother.

For each drug exposed child included in the study, the first child of the same gender born at Haukeland University Hospital on the same date, with a birth weight above the 10th percentile (≥ 3000 g), was invited to serve as a matched control. If they declined, the next child born on the same date (or the nearest date) was contacted. For the 20 opioid exposed children included, only 17 controls were successfully recruited, hence 20 exposed children and 17 control children under went MRI scanning. Eight children (five opioid exposed and three controls) were excluded from analyses due to abortion of the fMRI protocol by the child. In addition, scans from two opioid exposed children and two controls had to be excluded due to head movement artifacts (> 5 mm translation in any of the four experimental condi tions) and scans from two opioid exposed children were excluded due to dental braces distorting the images. Thus, the final sample for this study consisted of 11 prenatally opioid exposed children and 12 un exposed controls. Response logging failed for one participant (un exposed control) during fMRI. As scanner observational data revealed appropriate task performance, data from this participant was still in cluded in the analyses of the BOLD fMRI data, while analyses of task performance were run with $n = 11 + 11$.

All structural images were inspected by an experienced pediatric neuroradiologist. No major structural abnormalities were found. Somatic growth parameters (height, weight, and head circumference) were obtained prior to MRI scanning. Background and clinical char acteristics were obtained from medical records and/or questionnaires filled in by parents or foster parents. The prevalence of ADHD was determined by medical record review. Reports from earlier follow up of the 11 children in the opioid exposed group showed a mean intelligence quotient (IQ) score of 110.6 (SD: 13.9, median: 111, range: 82 130), as assessed by the Wechsler Intelligence Scale for Children, fourth edition and Wechsler Preschool and Primary Scale of Intelligence R.

The project was approved by the Regional Ethics Committee for Medical Research in Western Norway (REK Vest 2010/3301). Written consent was obtained from parents or foster parents and Child Welfare Services, as appropriate, for all participants. Written consent was also obtained from all children above the age of 12 years, and verbal consent from participants younger than 12 years.

2.2. fMRI task

A working memory selective attention task combining the n back task and the Stroop color word task was used (Braver et al., 1997; Stroop, 1935). The protocol of the present study has been used earlier by our group in a study on extremely preterm children. See ref. (Griffiths et al., 2013) for the complete description of the procedure. In short, the words RED, BLUE, GREEN, and YELLOW, each written in the three incongruent colors (e.g. red written in blue, green, or yellow), were presented sequentially through LCD goggles mounted on the head coil. The participants were asked to press a response key when either the word or the ink color of the word matched the one presented either 1 or 2 stimuli backwards in the presentation sequence, yielding four different experimental conditions (word 1 back, word 2 back, color 1 back, color 2 back). These four experimental conditions were presented in a pseudorandom order to avoid any order effects. A block design with alternating ON and OFF blocks was used, with four ON blocks and four OFF blocks in each of the four conditions. In each ON block, three to five target stimuli were randomly presented within a sequence of 16 stimuli in total, each presented for 2250 ms. All participants were in troduced to the procedure through a short computer program test

sampling all four research conditions, and effort was made to be sure the instructions were comprehended before entering the scanner.

2.3. MRI data acquisition

Structural and functional images were acquired on a GE Signa Excite HD 3.0 T (Milwaukee, WI, USA) MRI scanner. A high resolution three dimensional T1 weighted structural image was collected sagittally for co registration with functional data using a fast spoiled gradient re covery sequence (TR = 8 ms; TE = out of phase; FA 11°; 256 × 256 matrix; FOV = 256 mm; slice thickness 1.0 mm). Functional images were collected axially using an Echo Planar Imaging (EPI) sequence with the following parameters: TR = 3000 ms, TE = 30 ms, FA 90°, 128 × 128 matrix, FOV = 220 mm, no. of slices 38, slice thickness 3 mm with 0.5 mm skip, voxel size 1.72 × 1.72 × 3.5 mm. Fourteen EPI scans per 8 blocks, arranged in a task rest task manner, making a total of 112 scans, were analyzed for each of the four conditions (five initial dummy scans were discarded before data analysis). Total scan time was approximately 45 min.

2.4. Data analysis

2.4.1. Sample characteristics and task performance

For descriptive statistics, the mean and SD is reported, as well as counts and percentages. Although the two study groups were primarily 1:1 matched for sex and age, the groups were treated as independent in our analyses, as matching was disrupted by appropriate exclusions of more than one third of the participants as described in Section 2.1. Comparisons of continuous variables and dichotomous variables be tween groups were done with Gosset's independent *t* test and Fisher's exact mid p test respectively. During fMRI, the participants were in structed to respond to certain target stimuli, and time to correct answer was recorded. To allow for both response accuracy and reaction time to be modeled simultaneously, time to correct task response was analyzed using the Cox proportional hazards model (Cox, 1972). For each target stimuli, time to correct answer was registered, with maximal response time (2250 ms) registered if a correct response was not obtained. If there was not a correct answer, the time to response was considered to be censored as opposed to uncensored when the correct answer was obtained. Altogether, 1430 observations were included in these ana lyses (65 target stimuli × 22 children). As each child responded to multiple target stimuli, a frailty term for child was included (Hougaard, 1995). Primary exposure of interest was a group variable coded 0 for opioid exposed children and 1 for controls. The results are reported using the hazard ratio (HR) with 95% CI, e.g. a HR > 1 means a greater instant probability of a correct answer for a control than an exposed child. Other variables possibly influencing task performance were difficulty level (4 different experimental conditions) and birth weight. All children performed the same tasks, so by the design, ex perimental condition was independent of exposure group and was not adjusted for in the models. Birth weight may be a mediator of the effect of exposure on task performance, and analyses were done without and with birth weight as an additional covariate to study any mediating effect. Interactions were tested between group and both birth weight and difficulty level. All significance tests were two sided, and a sig nificance level of $p < 0.05$ was set. Analyses were performed using IBM SPSS Statistics version 23 and Stata version 14.0 (Stata Corp. College Station, TX).

2.4.2. MRI data

Image processing and data analyses were performed using the SPM12 software package revision 6470 (Welcome Trust Center for Neuroimaging, London, UK) and Matlab version 9.0 (MathWorks Inc., Natick, MA). Default preprocessing routines, as implemented in SPM12, were followed for realignment of EPI scans and co registration of the T1 weighted structural scan to the mean EPI scan in each of the four experimental conditions. Subsequent segmentation of the structural scan was performed, providing normalization parameters used to nor malize the EPI scans to Montreal Neurological Institute (MNI) space (resized voxels 3 × 3 × 3 mm). Finally, the EPI scans were smoothed with a Gaussian kernel of 8 mm. Visual inspection of all EPI scans was performed to assure quality.

Individual participant first level fixed effect analyses were per formed on the ON OFF block contrasts for the four experimental con ditions, creating four contrast images per person. These images were subjected to second level random effect analyses using the general linear model, as implemented in SPM12. Within group activation pat terns for the opioid exposed and control groups were modeled using one sample *t* tests, and two sample independent *t* tests were used to determine between group differences. To account for multiple com parisons a cluster extent, random field theory based family wise error (FWE) corrected threshold at $p < 0.05$ was used to define significant activations in all analyses, with a primary cluster defining threshold at $p < 0.001$. Anatomical location of significantly activated clusters was identified using Anatomical Automatic Labeling (Tzourio Mazoyer et al., 2002).

3. Results

3.1. Sample characteristics

Demographic and clinical characteristics of the 11 prenatally opioid exposed children and the 12 unexposed controls included in the final sample are shown in Table 1. Of note, there was a high prevalence of ADHD in the exposed group (64%), compared to the control group (8%). All opioid exposed children included in the study either lived in foster care homes or were adopted. These children had been placed outside their biological homes at a mean age of 19.6 months (SD: 21.4, range: 0 62). Four children in the exposed group were born to mothers undergoing OMT, whereas seven children were born to mothers with a history of heroin abuse during pregnancy. Prenatal exposure to drugs other than opioids was reported in 8 of 11 (72%) opioid exposed chil dren: benzodiazepines in six (55%), cannabis in three (27%), and am phetamines in three (27%). Six children in the exposed group were described with symptoms of NAS in the newborn period. According to questionnaires filled in by their mothers, none of the included controls were exposed to prenatal opioids or any other drugs.

Table 1
Sample characteristics for 11 prenatally opioid-exposed children and 12 unexposed controls.

Variable, *statistic*	Exposed group (n = 11)		Control group (n = 12)		p
		Range		Range	
Males, *n* (%)	6 (55)	n/a	6 (50)	n/a	0.842
Age at scan (months), *mean* (*SD*)	146.1 (13.3)	116–160	146.0 (10.6)	123–160	0.986
Head circumference (cm), *mean* (*SD*)	54.9 (1.4)	52.5–57.0	54.5 (1.7)	51.5–58.2	0.547
Height (cm), *mean* (*SD*)	153 (11.8)	127–169	150 (9.3)	130–167	0.560
Weight (kg), *mean* (*SD*)	43.9 (11.7)	22.8–64.5	43.4 (10.0)	29.3–68.8	0.906
Left handedness, *n* (%)	0 (0)	n/a	1 (8.3)	n/a	0.522
ADHD, *n* (%)	7 (64)	n/a	1 (8.3)	n/a	0.009
Birth weight (g), *mean* (*SD*)	2956 (520)	2300–4010	3545 (431)	3040–4200	0.007

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; n/a, not applicable; p = p values for group difference (controls vs. exposed) from independent *t*-test (continuous variables) and Fisher's exact test with mid-p correction (dichotomous variables).

Table 2
Results from Cox regression of time to correct response on a working memory-selective attention task in 11 prenatally opioid-exposed children and 11 unexposed controls.

Variable	Category	Unadjusted models			Adjusted for birth weight[b]		
		HR[a]	95% CI	p	HR[a]	95% CI	p
Group	Opioid-exposed	1.00	Reference	0.030	1.00	Reference	0.164
	Controls	1.46	(1.04, 2.06)		1.29	(0.90, 1.83)	
Birth weight group	2000–2500 g	1.00	Reference	0.002	1.00	Reference	0.010
	2500–3000 g	1.50	(0.81, 2.77)		1.50	(0.83, 2.70)	
	3000–3500 g	1.87	(1.20, 2.90)		1.62	(1.02, 2.57)	
	3500–4000 g	2.43	(1.40, 4.21)		1.89	(1.00, 3.56)	
	4000–4500 g	1.04	(0.60, 1.80)		0.87	(0.48, 1.55)	
Difficulty level	Color 1-back	1.00	Reference	< 0.001	n/i		
	Word 1-back	0.81	(0.70, 0.94)		n/i		
	Color 2-back	0.34	(0.28, 0.40)		n/i		
	Word 2-back	0.39	(0.33, 0.46)		n/i		

Abbreviations: HR, hazard ratio; CI, confidence interval; p = p-value for the variable from likelihood ratio test; n/i, not included.
[a] Estimated HR (instant probability of a correct answer) from Cox regression using a frailty model to account for dependency between multiple answers from the same participant.
[b] All children performed the same tasks, so by the design difficulty level was independent of exposure group and was not adjusted for in the model.

3.2. Task performance

Overall, mean response accuracies of 86.7% (SD: 7.85%) and 92.5% (SD: 7.89%) were found in the opioid exposed and control groups respectively, when the results from all the four experimental conditions were combined. Results from Cox proportional hazards models, used to analyze task performance, are presented in Table 2. The opioid exposed group responded slower, with fewer correct answers than the control group, as shown in Fig. 1, with an unadjusted hazard ratio (HR) of control vs. exposed = 1.46 (95% CI: 1.04 to 2.06; $p = 0.030$). Adjusting the model for birth weight revealed no significant group differences (HR = 1.29; 95% CI: 0.90 to 1.83; $p = 0.164$). Children with birth weights in the range 3000 4000 g performed better, with faster correct responses compared to children in the lowest birth weight group (< 2500 g). No significant interaction between group and birth weight was found. As expected, there were significant differences between the four experimental conditions ($p < 0.001$), with lowest ratio of correct responses in the more cognitive demanding 2 back tasks (Table 2). However, the interaction between group and difficulty level (experimental condition) was not significant ($p = 0.170$).

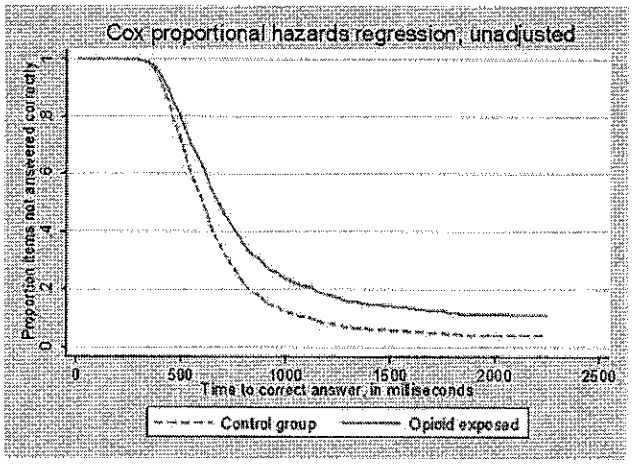

Fig. 1. Proportion of items not answered correctly in the working memory-selective attention task for 11 prenatally opioid-exposed children and 11 control children combined for four experimental conditions as estimated by a Cox proportional hazards model with frailty to account for dependency between multiple answers from the same child. Altogether 1430 tasks were analyzed showing higher proportion of items not answered correctly in the exposed group ($p = 0.030$).

3.3. fMRI activation patterns

Overall, the within group analyses showed similar, bilateral prefrontal and parietal areas of BOLD activation in both groups, but more widespread, diffuse activation in the exposed group in the more cognitive demanding conditions (word 2 back and color 2 back tasks). In the exposed group, only one large cluster, expanding widespread, bilateral cortical areas, survived corrections for multiple comparisons in each of these 2 back tasks. Fig. 2 shows within group activation patterns for all four tasks (word 1 back, word 2 back, color 1 back, and color 2 back) for the two groups. The corresponding MNI coordinates for peak voxel activations for the significant clusters are listed in Table 3.

Results from the between group analyses revealed increased activation in the exposed group in both 2 back conditions, whereas there were no significant group differences in the easier 1 back conditions. There were no areas where the control group showed increased activation relative to exposed children in any of the four experimental conditions (control minus exposed contrasts). In Fig. 3, clusters with significant group differences are shown, with corresponding peak voxel activations listed in Table 4. In the word 2 back condition, one significant cluster in the left prefrontal cortex, including the left precentral gyrus and superior and middle frontal gyrus, showed increased activation in the exposed group. Increased bilateral prefrontal activations in the exposed group were found in two clusters including left and right middle frontal gyri in the color 2 back condition.

4. Discussion

Results from this first fMRI study on prenatally opioid exposed children showed increased BOLD activation in prefrontal cortical areas in the exposed group, as compared to unexposed controls, during the most cognitive demanding versions of a working memory selective attention task. In both groups, task related activation patterns included parietal and prefrontal cortical areas previously shown to be important in working memory and selective attention tasks (Braver et al., 1997; Owen et al., 2005; Nee et al., 2007), a finding that supports the validity of our results. The opioid exposed group showed impaired task performance, but this group difference was no longer significant when analyses were adjusted for birth weight.

Aberrant brain activation patterns in children have been reported in fMRI studies exploring possible effects of maternal use of alcohol, cocaine, amphetamines, and tobacco on the developing fetal brain (Roussotte et al., 2010; Derauf et al., 2009; Bennett et al., 2013). However, to date, no study has examined specifically if prenatal opioid exposure affects brain activation patterns. The majority of functional

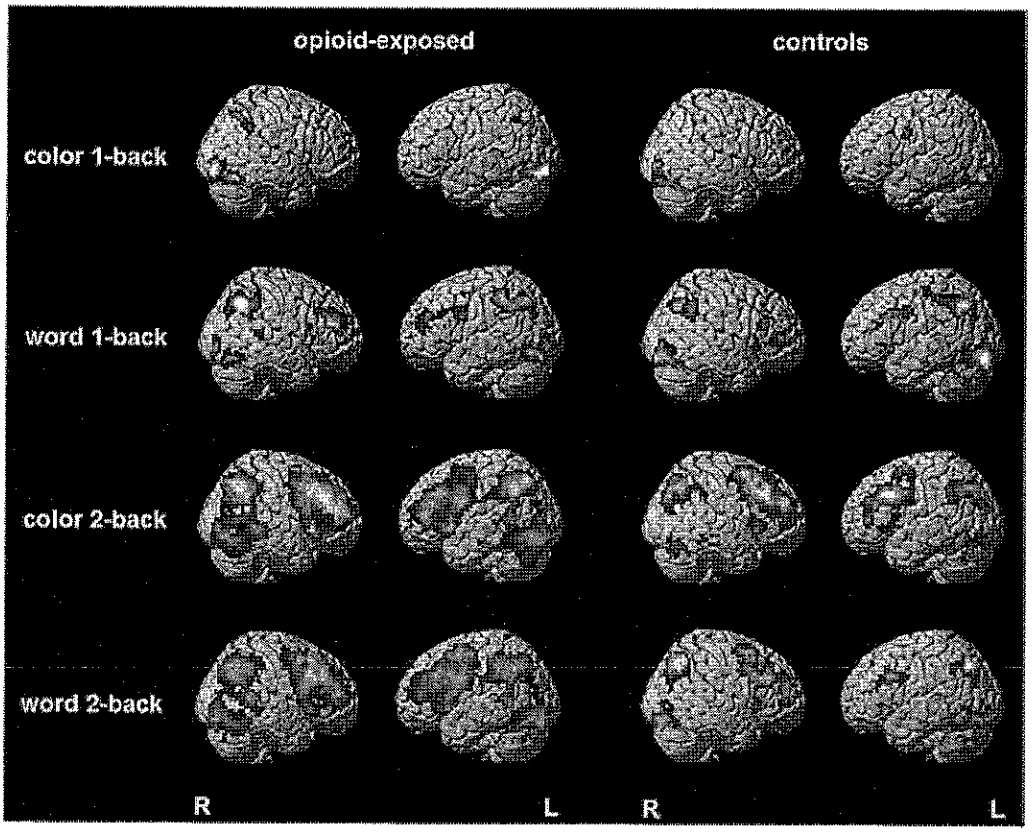

Fig. 2. Within-group BOLD activation patterns for the opioid-exposed group ($n = 11$) and the unexposed control group ($n = 12$) separated by the four experimental conditions. Surface renderings of activated clusters that survived corrections for multiple comparisons with a cluster-extent based threshold at FWE corrected $p < 0.05$. Abbreviations: BOLD, blood-oxygen-level-dependent; FWE, family-wise error; L, left; R, right.

brain imaging studies on prenatally drug exposed children, have reported on the effects of heavy prenatal alcohol exposure (Roussotte et al., 2010). Several of these studies have shown increased prefrontal BOLD response in alcohol exposed groups during working memory tasks, but with varying degrees of behavioral differences (Spadoni et al., 2009; Norman et al., 2013; O'Hare et al., 2009). Less efficient task related networks, or compensation for other less active regions, have been suggested as an explanation to these findings in alcohol exposed children (Spadoni et al., 2009; Norman et al., 2013). Similar compensatory mechanisms could possibly explain our finding of increased prefrontal activation in the opioid exposed group. On the other hand, Astley et al. (Astley et al., 2009) found significant working memory deficits in children with fetal alcohol spectrum disorders (FASD) with corresponding lower brain activation in extended prefrontal and parietal regions, particularly on the most cognitive demanding tasks. The authors have discussed a possible "ceiling effect", where the capacity of compensatory mechanisms to less efficient networks is surpassed, as an explanation to the inability of the FASD group to increase activity in response to increasing cognitive load (Astley et al., 2009). Impaired working memory performance with corresponding decreased prefrontal activation has also been reported in children exposed to methamphetamine (Roussotte et al., 2011). In the first BOLD fMRI study of adolescents with gestational cocaine exposure, no differences were found between exposed and unexposed groups in performance on a working memory task or BOLD activation patterns (Hurt et al., 2008). However, similar to our findings in opioid exposed children, Sheinkopf et al. reported increased prefrontal activation in a group of children with prenatal cocaine exposure compared to non exposed controls during a response inhibition task (Sheinkopf et al., 2009). On the other hand, reduced BOLD activity in frontal and cerebellar regions related to subtle attentional challenges were recently found in a group of adolescents with prenatal exposure to cocaine and/or heroin (Schweitzer et al., 2015). In young adults with histories of prenatal marijuana exposure, fMRI studies have shown increased brain activation across several different executive function tasks. However, unlike our findings in opioid exposed children, this increased activation has been consistently located in posterior brain regions (Smith et al., 2016).

Decreased activation in prefrontal cortical areas has been a consistent finding in numerous fMRI studies on children with ADHD (Dickstein et al., 2006). Reduced BOLD activity in frontal lobe regions in patients with ADHD relative to controls has been repeatedly reported across several different cognitive tasks (McCarthy et al., 2014). Less activity in frontoparietal networks involved in executive function has been one of the main findings in children with ADHD (Cortese et al., 2012). Contrary to this, we found increased prefrontal activation in our opioid exposed group, despite the fact that there was a very high rate of ADHD (64%) in this group. It is therefore tempting to speculate that these differences could reflect different neural correlates of ADHD in opioid exposed and non exposed groups. However, our sample was too small to allow for comprehensive statistical analyses of subgroups, like comparisons of opioid exposed children with and without a diagnosis of ADHD. Future studies including larger samples and control groups with and without ADHD would be needed to elucidate these possible differences.

Consistent with earlier reports of impaired executive function in children with prenatal opioid exposure (Konijnenberg and Melinder, 2015), the exposed group in the present study performed poorer on the executive function task compared to controls, with slower response and fewer correct answers. However, the group difference found in the unadjusted model was no longer significant when analyses were adjusted for birth weight. Birth weight as a mediator of the possible effect of opioid exposure on task performance could explain this finding. The

Table 3
Within-group analyses: Peak voxel descriptions for BOLD activation in significant clusters from whole brain analyses in 11 prenatally opioid-exposed children and 12 unexposed controls.

Task	Group	Anatomical area[a]	Cluster size[b]	Peak T[c]	Peak coordinates (MNI)		
					x	y	z
Color 1-back	Opioid-exposed	R Inferior occipital gyrus	329	4.80	33	88	7
		L Superior parietal gyrus	182	4.64	27	64	47
		L Inferior occipital gyrus	230	4.38	30	88	7
		L Supplementary motor area	146	4.09	3	14	50
		R Angular gyrus	133	3.73	39	64	50
	Controls	L Fusiform gyrus	163	4.43	42	82	16
		L Precentral gyrus	99	3.89	39	2	32
		R Middle occipital gyrus	106	3.74	39	88	1
Word 1-back	Opioid-exposed	R Middle frontal gyrus	1503	5.40	45	44	20
		L Parahippocampal gyrus	2985	4.99	33	43	1
		R Inferior occipital gyrus	84	4.82	33	88	4
		R Cerebellum	162	4.30	33	79	25
		L Precentral gyrus	98	3.73	48	11	32
	Controls	R Lingual gyrus	311	5.35	24	91	16
		L Fusiform gyrus	583	4.67	36	85	16
		L Middle frontal gyrus	1388	4.60	48	17	35
		R Middle frontal gyrus	919	4.22	39	44	8
		R Inferior parietal gyrus	312	4.14	51	49	41
Color 2-back	Opioid-exposed	R Middle frontal gyrus	19057	5.93	36	56	17
	Controls	L Middle frontal gyrus	4939	5.21	48	17	38
		L Superior occipital gyrus	778	5.17	18	64	38
		R Fusiform gyrus	137	4.59	48	31	16
		R Precuneus	848	4.50	30	52	29
		R Cerebellum	123	4.12	39	76	28
		L Thalamus	81	3.71	12	13	8
		L Cerebellum	149	3.71	45	70	28
		R Thalamus	119	3.70	12	10	11
Word 2-back	Opioid-exposed	R Superior frontal gyrus, dorsolateral	17,891	5.47	27	17	50
	Controls	R Angular gyrus	788	4.87	33	61	47
		R Lingual gyrus	273	4.74	24	88	16
		R Inferior frontal gyrus, triangular part	1082	4.62	39	29	23
		L Inferior parietal gyrus	454	4.61	30	70	41
		L Fusiform gyrus	331	4.23	39	73	16
		L Precentral gyrus	228	4.02	45	5	29
		L Supplementary motor area	218	4.00	3	20	50

Abbreviations: BOLD, blood-oxygen-level-dependent; L, left; MNI, Montreal Neurological Institute; R, right.

[a] Local maxima labeling from Anatomical Automatic Labeling.

[b] Cluster size in voxels (3 × 3 × 3 mm), only clusters that survived correction for multiple comparisons with a cluster-extent based threshold at family wise error (FEW) corrected $p < 0.05$ are shown.

[c] t-Values from one-sample *t*-tests.

exposed group in the present study had significantly lower birth weight compared to controls, and prenatal opioid exposure has been associated with increased risk of preterm birth and low birth weight (Mactier et al., 2014). However, there are myriads of risk factors associated with prenatal drug exposure, and it is still unclear if opioids have a direct, causal effect on these birth outcomes (Jones et al., 2014). It is therefore difficult to know whether birth weight should be conceptualized as a mediator or a confounder. A larger sample size or a more cognitive demanding task (e.g. a 3 back task) could possibly reveal more convincing group differences in task performance. However, previous studies (Griffiths et al., 2013), in scanner observations, and pilot scans performed prior to our study, have indicated that the 2 back tasks used were quite difficult for children in the chosen age range.

An abundance of data from animal and cell culture studies have demonstrated adverse effects of opioids on brain development that could possibly underlie altered brain activation patterns in prenatally exposed children (Hu et al., 2002; Dholakiya et al., 2016; Wu et al., 2014; Vestal Laborde et al., 2014; Farid et al., 2008). Interestingly both animal and human data have demonstrated reduced thickness of frontal cortical areas after prenatal opioid exposure (Walhovd et al., 2007; Sadraie et al., 2008). In animal models, the endogenous opioid system has been shown to be crucial in the control of oligodendrocyte function and myelination (Vestal Laborde et al., 2014), and interference with this system by maternal opioid use could alter the normal maturation process of the developing brain. There is also circumstantial evidence suggesting that opioids can alter myelination in prenatally exposed children (Walhovd et al., 2010; Walhovd et al., 2012). Incomplete myelination may result in poorer conduction efficiency and thus less efficient neural function in related networks.

There are several limitations to the present study. The results should therefore be considered preliminary and conclusions made with caution. First of all, an observational study design precludes firm conclusions about causality even if a causal relationship between prenatal opioid exposure and altered BOLD activation is plausible. The possible effect of prenatal opioid exposure on brain development cannot be distinguished from those of several known and unknown factors differing between the exposed and control groups, a challenge in all studies on drug exposed children. Some of the most obvious factors include genetic vulnerabilities and psychosocial and lifestyle factors associated with maternal substance abuse. The impact of many of these factors on brain activation patterns is largely unknown. However, some factors, like parental socio economic status, have been associated with altered BOLD activation, including increased prefrontal activation found in socioeconomically disadvantaged children (Sheridan et al., 2012). In our study sample, all opioid exposed children lived in stable family situations (either in foster care or adopted). Most of these children had been placed outside their biological homes at an early age. However, all had experienced multiple foster care placements. Social and environmental differences between our study groups could influence some of the observed differences. Future studies would benefit

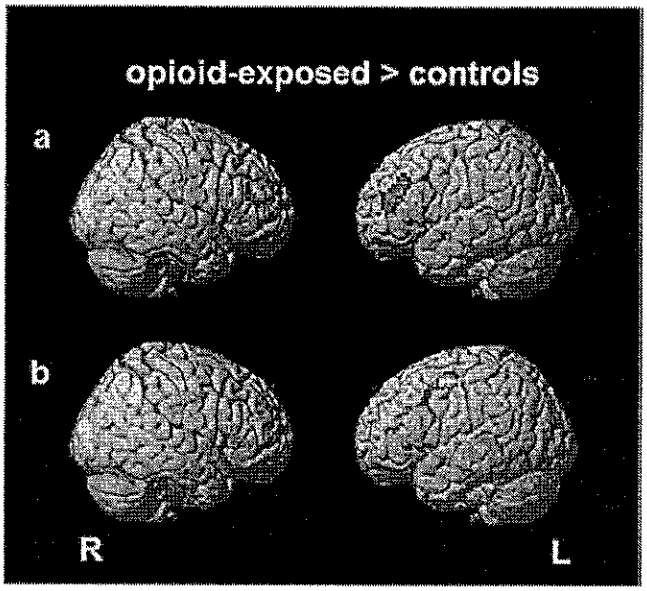

Fig. 3. Surface renderings of group differences in BOLD activation for the two most cognitive demanding experimental conditions with cluster-wise corrections for multiple comparisons (FWE corrected; $p < 0.05$). (a) color 2-back, (b) word 2-back. In both these conditions the opioid-exposed group ($n = 11$) showed increased BOLD activation relative to unexposed controls ($n = 12$). Abbreviations: BOLD, blood-oxygen-level-dependent; FWE, family-wise error; L, left; R, right.

from including control groups better matched for factors like living conditions, family income, and parental education.

A small sample size may have reduced our power to detect significant but subtle group differences and the ability to account for possible confounders. Most children in the opioid exposed group in our study were exposed to multiple drugs. Information regarding exposure to non opioid drugs was based on history without toxicology testing, and exposure may thus have been underestimated. Due to this uncertain degree of exposure and the small sample size, we were not able to control for exposure to non opioid drugs in our statistical modeling. Therefore, the possible influence of drugs other than opioids cannot be ruled out. However, only children with confirmed exposure to opioids, and no children with known exposure to heavy maternal alcohol consumption, were included. In addition, our study lacked reliable data for prenatal tobacco smoke exposure. However, contrary to our findings, Bennett et al. (Bennett et al., 2013) found greater prefrontal activation in their unexposed control group whereas tobacco exposed children showed greater activation in inferior parietal regions during an n back working memory task.

Due to the small sample size and the risk of masking possible opioid effects that were mediated by low birth weight, we did not attempt to adjust the between group analyses of BOLD fMRI data for birth weight. However, we find it unlikely that the selection of a low birth weight group should explain the increased prefrontal activation seen in our opioid exposed group, as decreased BOLD activation has been a consistent finding in fMRI studies of preterm and low birth weight groups, including one study using the same fMRI paradigm as the one used in the current study (Griffiths et al., 2013).

The use of a cluster extent based threshold to correct for multiple comparisons in our study precludes inferences about specific anatomical regions within significant clusters to be made with confidence (Woo et al., 2014). Even with a primary cluster defining threshold of $p < 0.001$, some of the activated clusters were large, spanning several anatomical regions. The anatomical labels for peak voxel activation listed in tables should therefore be interpreted with caution, as one cannot infer that all these peaks were truly activated, but only that there was a significant signal somewhere within each cluster. Detailed discussion and comparisons of the anatomical localization of BOLD activations could therefore not be performed based on our results. However, our main finding of increased activation in the exposed group was restricted to relatively small clusters that could be localized in prefrontal cortical areas with confidence. The effectiveness of cluster extent based thresholding to correct for multiple comparisons in fMRI studies has recently been called into question (Eklund et al., 2016), but the problem of inflated false positive rates was mainly shown for more liberal primary cluster defining thresholds than the one used in the present study ($p < 0.001$). To investigate differences in activation patterns in greater detail, larger samples allowing for voxel vise correction methods are needed.

The generalizability of our results is also limited, as the hospital based sample of prenatally opioid exposed children included in the study represents a subset of the exposed population. However, the signs of opioid effects on brain function in our sample warrant further research, and if possible population based samples should be included in future studies.

Finally, it should be acknowledged that fMRI uses level of oxygenated blood as a proxy for measuring the activity of neurons. The extent to which differences in the BOLD signal between our study groups represent actual differences in neuronal activation as compared to other possible underlying mechanisms, like altered vascularization, remains unknown.

5. Conclusion

Our findings suggest that prenatal opioid exposure affects brain activation patterns during a working memory selective attention task. Increased prefrontal activation in the exposed group in the most cognitive demanding tasks could represent compensatory mechanisms to less efficient task related networks. However, results should be considered preliminary until replicated in larger samples better suited to explore subtle differences and account for potential confounding factors.

Table 4
Between-group analyses: Peak voxel descriptions for BOLD activation in clusters with significant group differences between 11 prenatally opioid-exposed children and 12 unexposed controls.

Task	Contrast	Anatomical area[a]	Cluster size[b]	Peak 't'[c]	Peak coordinates (MNI)		
					x	*y*	*z*
Color 2-back	Opioid-exposed > controls	L Middle frontal gyrus	277	5.78	33	38	23
		R Middle frontal gyrus	186	5.17	33	41	17
Word 2-back	Opioid-exposed > controls	L Precentral gyrus	148	5.00	39	4	53

Abbreviations: BOLD, blood-oxygen-level-dependent; L, left; MNI, Montreal Neurological Institute; R, right.
[a] Local maxima labeling from Anatomical Automatic Labeling.
[b] Cluster size in voxels (3 × 3 × 3 mm), only clusters that survived correction for multiple comparisons with a cluster-extent based threshold at family wise error (FEW) corrected $p < 0.05$ are shown.
[c] t-Values from independent *t*-tests.

Transparency document

The Transparency document associated with this article can be found, in online version.

Disclosure

The authors have no financial or other conflicts of interest to dis close.

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